Morphological Characterization of NaCl-tolerant *Phaseolus vulgaris* Seeds

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ABSTRACT

Bean is a source of proteins but salinity is an agricultural limiting factor. Evaluation of the plant germplasm to identify a tolerant genotype is an adequate strategy. The present report is focused on the early stage of bean seed germination. Seeds of 89 cultivars were weighed after 5 days of growth in 0 or 270 mmol/L NaCl. The five heaviest cultivars were regarded as NaCl tolerant; and the five lighter, as susceptible. Ten fresh seeds per cultivar were scanned. The following indicators were measured: area, equivalent diameter, major axis length, minor axis length, eccentricity, convex area, solidity, and extent. The matrix obtained was used to estimate Fisher’s linear discriminant functions for susceptible and tolerant cultivars. Two variables were disregarded (convex area and solidity). Functions classified correctly 100% of tolerant or susceptible originally grouped cultivars. Fisher’s linear discriminant functions are important tools for bean breeders. Seeds from new bean genotypes can be scanned as described here. Data were evaluated in both discriminant functions. If the resulting value of the tolerant-discriminant function was statistically higher than that of the susceptible-discriminant function, then the new bean genotype can be regarded as putatively tolerant. In addition to the practical use of the assay described here, we reached two unexpected conclusions. Firstly, bean seeds were not so NaCl-sensitive during early stages of germination: we observed radical emergence with a high concentration (360 mmol/L). Secondly, the larger the bean seed size, the lower the sensitivity to NaCl.

Keywords: area, axis length, bean, convex area, diameter, eccentricity, equivalent solidity, extent, Fisher’s linear discriminant functions, MATLAB, salinity

INTRODUCTION

Common bean is an important source of proteins in developing countries (Durante and Gius 1997). It is sensitive to salts, like many other leguminous crops (Maas and Hoffman 1977). Soil salinity is an increasingly severe agricultural problem in several world regions (Shannon 1986), e.g. in a narrow island such as Cuba. The evaluation of genetic variability of plant germplasm to identify a tolerant genotype has been one of the most frequent strategies to overcome this problem (Kingsbury and Epstein 1984).

Characters such as yield, survival, vigour, leaf damage, and plant height, have been the most commonly used criteria for identifying salinity tolerance (Maas and Hoffman 1977; Shannon 1984). Other indices of tolerance have been proposed that are based on specific physiological characteristics, for instance, accumulation of specific ions in shoots or leaves, or the production of a specific metabolite (Shannon 1986).

Salinity tolerance, however, is usually assayed in terms of absolute or relative growth or yield (Maas and Hoffman 1977; Shannon 1984). This is largely due to ease of measurement and because, in the end, yield (both absolute and relative) under saline conditions is usually the ultimate goal (Bayuelo-Jiménez et al. 2002).

Evaluation of salt tolerance in legumes has been attempted by a variety of cultural techniques with plant material ranging from germinating seeds to seedlings to mature plants (Prisco and O’Leary 1972; Keating and Fisher 1985; Subbarao et al. 1991; Ortiz et al. 1994; Ferri et al. 2000; Lopez et al. 2002; Jungklang et al. 2003; Aroca et al. 2007; Murillo-Amador et al. 2007; Webber et al. 2008; Rogers et al. 2009; Priyanka et al. 2010). Evidence collected from various species suggests that salt tolerance is a developmentally regulated, stage-specific phenomenon, so that tolerance at one stage of development may not be correlated with tolerance at other developmental stages (Shannon 1986; Bayuelo-Jiménez et al. 2002).

The present report is only focused on the early stage of bean seed germination. It describes the generation of Fisher’s linear discriminant functions for morphological identification of NaCl-tolerant bean seeds. To our knowledge, the relationship between the bean seed size and its tolerance to sodium chloride has not been published to date.

MATERIALS AND METHODS

Seeds of 89 bean cultivars were provided by the Cuban National Institute for Agricultural Sciences (INCA, Havana, Cuba). One of these cultivars was randomly selected to test the effect of sodium chloride on seed germination. Petri dishes (ø: 15 cm, height: 2.5 cm) with filter papers (Whatman, single layer) were used. Five concentrations dissolved in distilled water were compared (0, 90, 180, 270, 360 mmol/L). Each dish contained 10 seeds and 15 mL solution. Fresh plantlet mass was recorded after 5 days of incubation in the dark. In a second experiment, the effect of two concentrations of NaCl (0, 270 mmol/L) on the whole bean collection was evaluated as described above.

The five heaviest cultivars were regarded as NaCl-tolerant; and the five lighter, as susceptible. Ten fresh seeds per cultivar were simultaneously scanned (jpg format, 600 dpi color image, Canon F189200 ImageRunner 1023N, Cannon IR Toolbox 4.9). The following eight indicators were measured from the scanned image by MATLAB (version 7.0.1.247014 (R14), Service pack 1; September 13, 2004): area, equivalent diameter, major axis length, minor axis length, eccentricity, convex area, solidity, and extent.
RESULTS AND DISCUSSION

NaCl reduced seed germination (Fig. 1). Fig. 2 shows the results of the most contrasting members of the germplasm collection. Cultivars 28, 35, 3, 69 and 64 germinated faster than 87, 83, 24, 82 and 84, in both NaCl-free and NaCl-containing Petri dishes (Fig. 2A, 2B).

The scanned image is shown in Fig. 3 and the eight morphological indicators measured appear in Fig. 4 and 5. Table 1 shows extreme values observed.

The statistical package-generated discriminant functions are shown in Fig. 6. Two variables were excluded: convex area and solidity. Requirements of this kind of analysis were met. Groups of the dependent variable were mutually exclusive: cultivars had been previously classified as tolerant or susceptible (Fig. 2B, 3). Therefore, the dependent variable was not metrical but categorical. Independent variables were all metrical. The number of cases (100) was higher than twice the number of variables (8).

Repeated observations of each cultivar of the original matrix were evaluated in these discriminant functions to test the accuracy of the functions obtained (Table 2). Functions classified correctly 100% (10 bean cultivars) of tolerant or susceptible originally grouped cultivars (Fig. 2B).

The Fisher’s linear discriminant functions shown in this paper (Table 3) are important tools for those bean-breeding programs focused on the production of salinity tolerant plants. Seeds from new bean genotypes can be scanned as described here. Data are evaluated in both discriminant functions. If the resulting value of the tolerant-discriminant function is statistically higher than that of the susceptible discriminant function, then the new bean genotype can be regarded as putatively tolerant. Although the new genotype tolerance still requires additional confirmation under field environment, the results described here allow some research cost reductions because there is no inclusion of a large number of susceptible cultivars.

In addition to the practical use of the assay described

Table 1 Maximal and minimal values (pixels) recorded in the experiment and used for data standardization according to Kantardzic (2003).

<table>
<thead>
<tr>
<th>Bean cultivar</th>
<th>Area</th>
<th>Equivalent diameter</th>
<th>Major axis length</th>
<th>Minor axis length</th>
<th>Eccentricity</th>
<th>Convex area</th>
<th>Solidity</th>
<th>Extent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal value recorded</td>
<td>76876</td>
<td>312860293</td>
<td>433776876</td>
<td>231143471</td>
<td>0.880834</td>
<td>79445</td>
<td>0.99136</td>
<td>0.82889</td>
</tr>
<tr>
<td>Minimal value recorded</td>
<td>20705</td>
<td>162365097</td>
<td>197904497</td>
<td>126049564</td>
<td>0.675773</td>
<td>21034</td>
<td>0.95358</td>
<td>0.60373</td>
</tr>
</tbody>
</table>
here, we reached two unexpected conclusions. Firstly, bean seeds were not so NaCl-sensitive during early stages of germination. Most experiments to screen tolerance to sodium chloride used < 150 mmol/L, e.g. Meloni (2003) with cotton and Atia et al. (2006) with Crithmum maritimum seeds. In contrast, we observed radical emergence with 360 mmol/L. Our second and more important conclusion was: the larger the bean seed size, the lower the sensitivity to sodium chloride. Larger seeds contain more hydrophilic compounds to make imbibition possible (Winn 1985; Vleeshouwers et al. 1995; Vaughton and Ramsey 1998; Leishman et al. 2000; Cordazzo 2002; Parciak 2002; Dyer 2004; Van Mölken et al. 2005). At present, bean cultivars with potential salinity tolerance are being evaluated in different marginal farming communities of Ciego de Avila (Cuba).

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REFERENCES


Fig. 4 Morphological indicators of bean seeds.
Fig. 5 Summarized morphological indicators of bean seeds.


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